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Immuno-effector characteristics of peritoneal cells during CAPD treatment: A longitudinal study

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Immuno-effector characteristics of peritoneal cells during CAPD treatment: A longitudinal study. Peritoneal cells (PC) from 75 patients were immuno-phenotypically and functionally characterized during the first year of CAPD treatment (PCcapd) and compared to PC obtained by laparoscopy of healthy women (control peritoneal cells). Patients were divided, according to their peritonitis incidence (PI), into a high PI (HPI) and a low PI group (LPI). The yield of PCcapd decreased significantly over the year. The differential cell count and immunophenotype of PCcapd remained unchanged in the LPI group, but the percentage of macrophages decreased over the year in the HPI group. Macrophages in the PCcapd, when compared to control peritoneal cells, had a less mature phenotype as measured by RFD7 expression but a higher Fc-receptor expression. The PCcapd showed a higher percentage of B cells, CD4 positive T cells and activated T cells bearing HLA-DR/DQ when compared to the control peritoneal cells. Over the year a decrease in chemotactic activity of the PCcapd towards 10^{-8} M N-formylmethionyl-leucyl-phenylalanine and dialysis effluent was observed in LPI patients but not in HPI patients. After one year of treatment, a significantly higher percentage of phagocytosing macrophages in the PCcapd of HPI patients was found when compared to LPI patients. During the year there was an increase of immunophagocytosis of PCcapd independent of PI. In conclusion, the CAPD peritoneal cellular immune system showed signs of both immaturity and activation. The decrease in the yield and in the chemotactic activity of PCcapd suggests an adaptation to the chronic stimulus of the dialysis fluid. The higher chemotactic and phagocytosing activity of the PCcapd after one year of CAPD treatment indicate that the peritoneal cells of HPI patients are more activated compared to LPI patients.

Peritoneal macrophages from CAPD patients have been reported to be relatively immature cells [1, 2] when compared to control peritoneal macrophages. Characteristics of CAPD peritoneal macrophages such as increased respiratory burst activity, increased Fc-receptor expression, morphology of peroxidase staining pattern and increased killing of *Candida albicans* blastospores also suggests that these cells are functionally activated [1–5]. These studies on the immune system of the CAPD peritoneal cavity are different in methodology and are therefore difficult to compare. For instance, there are striking differences in the reports on the percentage of macrophages in the CAPD peritoneal cells, ranging from an average of 40% to 90% [1, 2]. Furthermore, most studies undertaken so far have been cross sectional in design and do not account for the possible confounding influence of duration of CAPD treatment on the immunophenotype and functional aspects of the CAPD peritoneal cells.

Both the immaturity and activity of the CAPD peritoneal cells could have clinical implications. An immature, not well-organized immune system may contribute to an increased susceptability to bacterial peritonitis.

A decreased secretion of fibronectin and IL-1, an increase in prostaglandin E_2 production and a decrease in bacterial killing capacity of peritoneal macrophages have been related to a high peritonitis incidence in CAPD patients [6, 7]. An activated immune system could contribute to the fibrosis of the peritoneal membrane and loss of ultrafiltration that is observed during long term CAPD treatment via mediators such as IL-1β and TNFα [8–11].

Few studies have investigated the immunophenotype of the peritoneal cells from CAPD patients in relation to peritonitis incidence. Davies et al [1] showed a relation between a high peritonitis incidence and low RFD7 (a maturation marker) expression on peritoneal macrophages and a low expression of HLA-DR on peritoneal lymphocytes. Two previous studies showed a relation between a low percentage of mesothelial cells (MC) and a high peritonitis incidence [5, 12].

The present study has been designed to study the following questions: (1) is the CAPD peritoneal cellular immune system immature or activated; (2) is this influenced by duration of CAPD treatment; and (3) are the characteristics of the CAPD peritoneal cellular immune system related to the incidence of bacterial peritonitis.

For this purpose the cellular composition, immunophenotype, chemotaxis, Fc-receptor positivity and immunophagocytosis of CAPD peritoneal cells were investigated during the first year of CAPD treatment. The data were compared with peritoneal cells obtained by laparoscopy of healthy women (control peritoneal cells).

Methods

Patient population

From January 1987 to September 1991, 75 CAPD patients were studied. This was a random selection of the 101 patients that had started CAPD in this period. Nineteen out of the 75

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Table 1. Clinical data of CAPD patients

	Number of patients
Male/female	41/34
Age *years*	
15–35	15
36–55	23
56–75	37
Underlying kidney disease	
Chronic glomerulonephritis	17
Diabetes mellitus	12
Chronic pyelonephritis	5
Polycystic kidney disease	7
Obstructive uropathy	3
Hypertension	13
Various	8
Unknown	10
Catheter system	
Standard	51
Disconnect	24

patients did not complete the first year of treatment; 5 patients were transferred to hemodialysis, 4 patients had a kidney transplantation and 10 patients died (one case related to peritonitis). The clinical data of the 75 patients are shown in Table 1. Patients were divided into two groups according to their peritonitis incidence (PI): a high PI (HPI patients, PI > 1 episode a year) and a low PI (LPI patients, PI ≤ 1 episode a year) group.

The 19 patients who dropped out were treated with CAPD on average for 6.0 months (median: 6.0, range: 2.0 to 10.0). Their median PI (calculated as number of peritonitis episodes per patient/month) did not differ significantly from the 56 other patients.

Cell populations

Peritoneal cells were obtained from the overnight dialysate (dwell time 8 to 10 hr) effluent. The complete dialysate was drained into 50 ml tubes (Greiner, Alphen a/d Rijn, The Netherlands) and cells were isolated by centrifugation at 200 × g for 10 minutes at 4°C. The supernatant was discarded, the cell pellets combined and centrifuged at 200 × g for 10 minutes at 4°C. The cells were washed once in 50 ml RPMI 1640 and the cell pellet resuspended in 1 to 2 ml immunoglobulin free αRPMI (RPMI 1640 medium supplemented with 2 mM L-glutamine, 50 U/ml penicillin and 50 μg/ml streptomycin; Mycofarm, Delft, The Netherlands), and 10% heat-inactivated immunoglobulin-free fetal calf serum (Gibco, Bio-Cult, Irvine, UK) and kept on ice until use. All patients used commercial dialysis fluid Dianeal^{R} (Baxter) with various glucose concentrations. The peritoneal dialysis effluents (PDE's) were analyzed every two to three months during the first year of treatment. All PDE's were analyzed at least one month after an episode of peritonitis.

The peritoneal cells obtained from healthy women undergoing laparoscopic sterilization were used as a control peritoneal cells population. Patients with endometriosis, pelvic inflammatory disease, or neoplasia were excluded because of reported macrophage abnormalities [13]. Peritoneal fluid (10 to 15 ml) was aspirated by gentle suction from the cul-de-sac and anterior uterovesical compartment [2]. This procedure yielded a median of 8.7 × 10^6 cells (range: 0.8 to 46.0 10^6 cells, Fig. 1).

All cell samples were counted in a Bürker counting chamber and viability was always above 90%. Differential cell counts were done on cytocentrifuge preparations stained with May-Grünwald Giemsa (MGG) and at least 200 cells were counted.

Peritonitis incidence (PI) was calculated as the number of peritonitis episodes (peritoneal cell count > 100 × 10^6/liter and more than 50% neutrophilic granulocytes) in the first year of treatment. Micro-organisms were cultured from the peritonitis peritoneal effluent as described previously [14]. The PI was calculated only in patients that had completed the first year of treatment (N = 56). The median PI was 1.0 (range, 0 to 5; mean, 1.2) peritonitis episodes per patient (Fig. 2).

Monoclonal antibodies

For the recognition of monocytes and macrophages monoclonal antibody (McAb) EBM-11 (pan macrophages marker, Dakopatts, Glostrup, Denmark) [15, 16] and RFD7 (recognizing mature macrophages; gift of Dr. L.W. Poulter) [17] were used. Anti-CD22 (pan B cell marker; Dakopatts), anti-CD2 (pan T cell marker; Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands), anti-CD8 (recognizing cytotoxic/suppressor T cells; Sanbio, Uden, The Netherlands) and anti-CD4 (recognizing helper/inducer T cells; Sanbio) were used to identify the different subsets of lymphocytes. For the determination of the percentage of HLA-DR/DQ positive cells McAb produced by clone 9.3F10 (ATCC, Rockville, New York, USA) [18] was used.

Immunocytochemistry

Cytocentrifuge preparations of peritoneal cells were fixed in phosphate buffered formalin/aceton, washed twice in phosphate buffered saline with 0.5% bovine serum albumin (PBS+) and incubated for 45 minutes with the different McAb's. After washing twice in PBS+ they were incubated for 45 minutes with horseradish peroxidase-conjugated rabbit anti-mouse IgG (Dakopatts) diluted in PBS+ with 1% pooled human serum. After washing twice in PBS peroxidase activity was shown by incubation with diaminobenzidinetetrahydrochloride (0.5 mg/ml, DAB), imidazole (3.4 mg/ml) and H_2O_2 (0.03%). Control preparations showed no staining with an irrelevant antibody. A minimum of 200 cells were examined for the presence of the different antigens.

Chemotaxis

Chemotaxis was performed in a 48-well microchemotaxis chamber [19]. Twenty-five microliters of RPMI 1640 (control) and 10^{-8} M N-formylmethionyl-leucyl-phenylalanine (FMLP) in RPMI 1640 were placed in the wells of the bottom chamber. A chemo-attractant has been shown in the peritoneal dialysis effluent (PDE) but not in the fluid obtained by laparoscopy [20]. Therefore, the PDE from which the CAPD peritoneal cells were obtained was also tested for its chemo-attractivity. A polycarbonate filter sheet (Nucleopore Inc., Cabin John, Maryland, USA), polyvinylpyrrolidone-free (PVP-F), 10 μm thick and 8 μm pore size was placed over the wells. A PVP-F filter was used to prevent dropping off of migrated peritoneal cells from the lower filter surface [21]. Peritoneal cells adjusted to 2.0 × 10^4 cells/50 μl αRPMI (RPMI 1640 medium supplemented with 2 mM L-glutamine, 50 U/ml penicillin and 50 μg/ml streptomycin (Mycofarm, Delft, The Netherlands), and 10% heat-inactivated fetal calf serum (Gibco, Bio-Cult, Irvine, UK) were added to each top well and the chambers were incubated in moist air

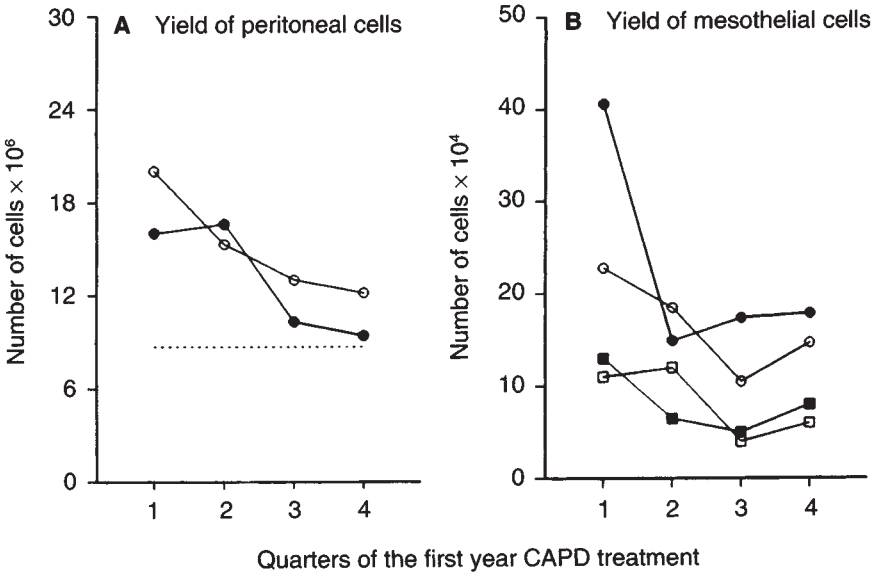

Fig. 1. *The total yield of CAPD peritoneal cells (A) and the total yield of mesothelial cells (B).* The open symbols represent the high peritonitis incidence group (N = 19) and the closed symbols the low peritonitis incidence group (N = 37). Male (●, ○) and female (■, □) CAPD patients are shown separately in Figure 2B. The dashed line represents the laparoscopy cells.

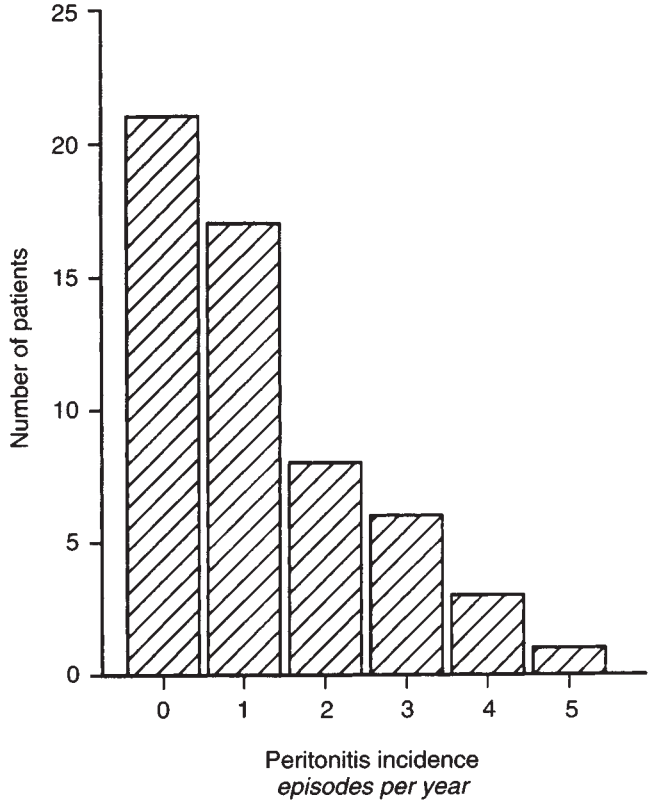

Fig. 2. *The distribution of the number of peritonitis episodes per patient in the first year of CAPD treatment* (N = 56).

with 5% CO_2 at 37°C for three hours. Each test was performed in triplicate. After incubation, the chamber was disassembled and the filter removed. Nonmigrated cells were wiped off and the filter was fixed in methanol and stained with Coomassie Brilliant Blue. The number of cells per 0.36 mm^2 area was counted manually using a scored eyepiece. For each well 25 areas were counted and the average used to determine the number of migrated cells in the area available (8 mm^2). The values are expressed as the percentage of migrated cells of the total number of cells in the upper well.

Freshly isolated monocytes were used as a standard cell population. Peripheral blood mononuclear cells (PBMC) were isolated on a Lymphoprep gradient (Nycomed, Oslo, Norway) from 10 ml heparinized peripheral blood. Using a MGG staining on a cytocentrifuge preparation the percentage of monocytes was determined and the PBMC adjusted to 4.0 × 10^5 monocytes/ml.

Detection of Fc-receptor and immunophagocytosis

The percentage of Fc-receptor-positive peritoneal cells was determined by rosette formation with IgG-coated sheep red blood cells ([IgG]SRBC) using routine procedures [22]. SRBC were washed and a 1.6% (vol/vol) suspension of packed cells in PBS+ was made and rabbit anti-SRBC IgG (Nordic, Tilburg, The Netherlands) to a subhemagglutinating concentration added. After incubation for 30 minutes at 37°C the SRBC were washed twice and resuspended in IgG free αRPMI to a 1.6% (vol/vol) suspension. Two hundred microliters of [IgG]SRBC (1:10 diluted) suspension was added to 200 μl of peritoneal cells suspension (1 × 10^6/ml in IgG free αRPMI). After a short centrifugation (for 5 min at 50 g and 4°C) followed by a one hour incubation at 4°C the percentage [IgG]SRBC binding peritoneal cells was microscopically determinated. Binding by peritoneal cells of three or more erythrocytes per cell was considered positive.

Immunophagocytosis was performed by the addition of 25 μl of peritoneal cells suspension (1 × 10^7/ml in IgG free αRPMI) to 25 μl of the [IgG]SRBC suspension. After one hour of incubation at 37°C the extracellular red cells were lysed osmotically, and after washing of the peritoneal cells cytocentrifuge preparations were made and MGG stained. The percentage of phagocytosing macrophages and the number of [IgG]SRBC per phagocytosing macrophages ([IgG]SRBC/macrophages) were determined. The phagocytosis capacity was calculated as: yield

Table 2. Micro-organisms isolated from the peritonitis effluent

	Low peritonitis incidence					High peritonitis incidence				
Quarters of the year	1	2	3	4	Total	1	2	3	4	Total
Peritonitis episodes	7	3	4	2	16	18	10	15	11	54
Organism										
Gram positive	6	2	3	1	12	14	9	10	10	43
S. epidermidis	4	1	3	1	9	7	7	6	6	26
S. aureus	2	1	—	—	3	7	2	3	4	16
Micrococcus sp.	—	—	—	—	—	—	—	1	—	1
Gram negative	1	1	1	1	4	2	2	3	1	8
Pseudomonas sp.	—	—	—	1	1	1	1	—	—	2
Streptococcus sp.	1	—	—	—	1	1	—	1	1	3
Klebsiella sp.	—	—	—	—	—	—	—	2	—	2
E. coli	—	1	—	—	1	—	—	—	—	—
Acinetobacter sp.	—	—	1	—	—	—	—	—	—	—
Citrobacter sp.	—	—	—	—	—	—	1	—	—	1
No organism cultured	—	—	—	—	—	—	—	1	2	3

of peritoneal cells × percentage of phagocytosing macrophages × [IgG]SRBC/macrophages.

Statistical analysis

All parameters were analyzed for their relation with duration of CAPD and PI. The first year of CAPD treatment was divided into quarters: 1 to 3 months, 4 to 6 months, 7 to 9 months and 10 to 12 months after starting CAPD. Data of all patients studied were expressed as medians with the range for each study quarter. Trend analysis was performed using unbalanced repeated measures models with structured covariance matrices (BMDP Statistical Software) [23]. For comparing CAPD peritoneal cells with control peritoneal cells the Wilcoxon test for unpaired data was used. Correlation between different parameters was analyzed using the Spearman rank correlation test.

Results

Patient population

Clinical parameters (Tables 1 and 2) were analyzed for their correlation to all measured parameters. Women had a significantly lower percentage and yield of MC in their effluent compared to men at every quarter of the year ($P < 0.03$, Fig. 1). The clinical data (including the isolated organism as shown in Table 2) of the 56 patients who completed the first year of treatment did not differ significantly between the two PI groups. The immunological parameters of the peritoneal cells of CAPD patients without a peritonitis episode during the first year of treatment were not statistically significant different from the immunological parameters of the peritoneal cells of CAPD patients with one peritonitis episode.

Yield of peritoneal cells and differential cell count

During the first year of CAPD treatment there was a gradual decrease in the yield of CAPD peritoneal cells (from 16.4×10^6 to 10.7×10^6 cells; Fig. 1). In contrast to the control peritoneal cells, the CAPD peritoneal cells showed a lower percentage of macrophages throughout the year (P values 0.04, 0.06, 0.2 and 0.04 for each quarter, respectively) and MC comprised 1 to 2% of the CAPD peritoneal cells. No MC were detectable in the control peritoneal cells. The MC could be easily recognized as round cells with a dark staining cytoplasm and large round nuclei with easy visible nucleoli, as shown previously [12]. The MC showed signs of a stimulated peritoneum, since many MC had multiple nuclei, were irregular in size and often were lying in large aggregates without a regular structure.

The differential cell count of peritoneal cells of HPI patients, but not of LPI patients, showed an increasing trend for the percentage of lymphocytes and a decreasing trend for the percentage of macrophages ($P < 0.01$, Fig. 3). The percentages of granulocytes in HPI and LPI patients were similar and remained unchanged over the year.

Immunophenotype

Macrophages. Macrophages in the CAPD peritoneal cells and control peritoneal cells were strongly HLA-DR/DQ positive. The control peritoneal cells contained more EBM-11 positive cells (84.5%, range 46.5 to 93.0%) that had a higher expression of RFD7 (31.5%, range 14.5 to 56.5%) when compared to the CAPD peritoneal cells of both the LPI and HPI patients ($P < 0.01$). A decreasing trend over the year of the percentage of cells reactive for the macrophage marker EBM11 was observed in HPI patients ($P < 0.01$, Fig. 4).

T cells. In general the CAPD peritoneal cells contained more CD2 positive cells, but this difference was only statistically significant in the first quarter ($P = 0.03$). Both cell populations had a CD4/CD8 ratio below 1.5, but the CAPD peritoneal cells contained a relatively higher percentage of CD4 positive T cells ($P < 0.01$, 0.06, 0.13 and 0.03, respectively) that sometimes exceeded the number of CD8 positive cells more than tenfold. Therefore the CD4/CD8 ratio of the CAPD peritoneal cells was generally higher, reaching a statistically significant difference in the first ($P = 0.04$) and fourth quarter ($P = 0.03$). The percentage of HLA-DR/DQ positive cells exceeded the percentage of macrophages plus the percentage of B cells. This confirmed the observation of many CAPD peritoneal T lymphocytes bearing HLA-DR/DQ [24]. The percentage of CD2, CD4, CD8 positive lymphocytes showed no relation with the PI.

B cells. The control peritoneal cells contained very few B cells as compared to the CAPD peritoneal cells (0.2% vs. 1.5%, $P < 0.01$ for every quarter). The percentage of CD22 positive lymphocytes showed no relation with the PI.

Chemotaxis

The percentages of CAPD peritoneal cells migrating to FMLP (from 6.8% to 4.3%, $P = 0.03$) and PDE (from 7.4% to 5.3%, P

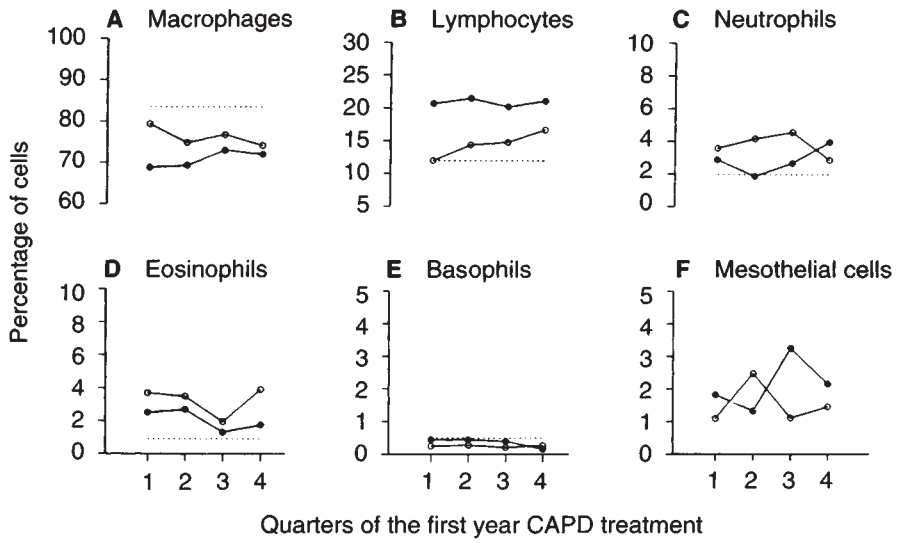

Fig. 3. *The differential cell count of the CAPD peritoneal cells.* The open symbols represent the high peritonitis incidence group (N = 19) and the closed symbols the low peritonitis incidence group (N = 37). The dashed line represents the laparoscopy cells.

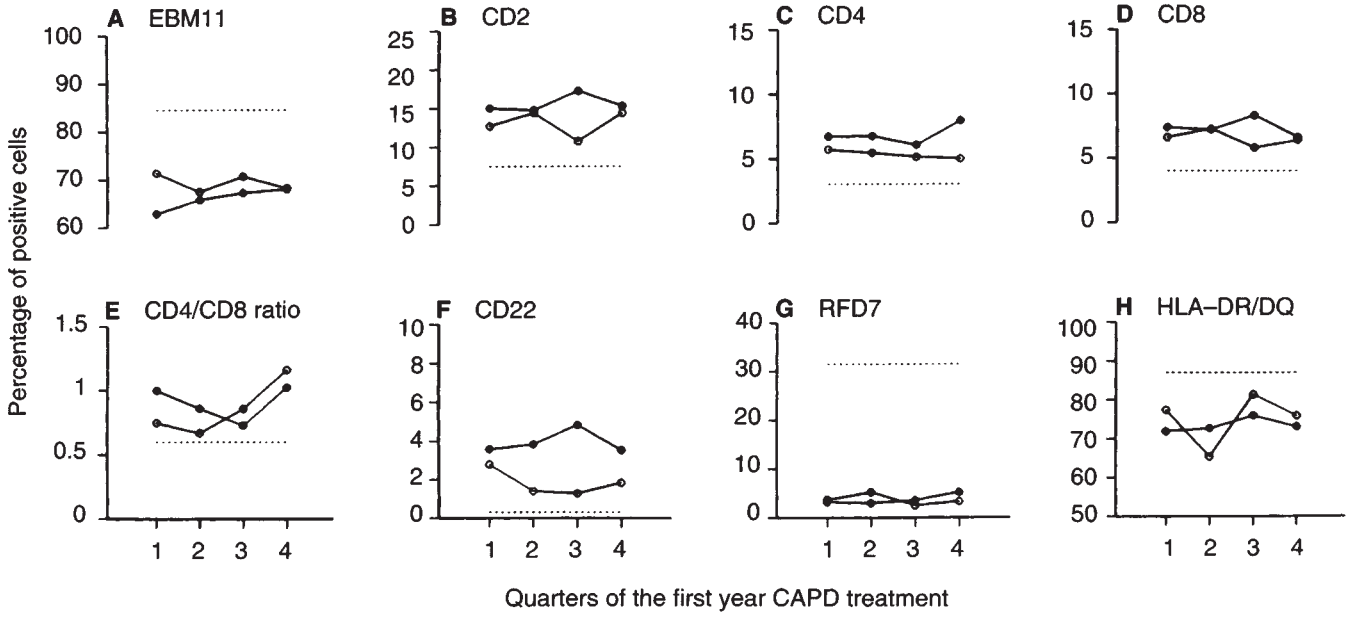

Fig. 4. *The percentages of CAPD peritoneal cells reactive with the different monoclonal antibodies.* The open symbols represent the high peritonitis incidence group (N = 19) and the closed symbols the low peritonitis incidence group (N = 37). The dashed line represents the laparoscopy cells.

= 0.02) decreased over the year in LPI patients (Fig. 5). The difference in chemotactic response to FMLP between the two PI groups increased during the year and was statistically significant in the fourth quarter (4.3% vs. 10.1%, $P < 0.05$). The CAPD peritoneal cells of LPI patients tended to have a lower response to FMLP than the control cells ($P < 0.05$ in the third quarter). The chemotactic response of CAPD peritoneal cells and control peritoneal cells to the control medium RPMI 1640 (median 2.5%) was similar but was not related to duration of CAPD or PI. The percentage healthy donor monocytes migrating to PDE was similar during the first (17.9 ± 7.8%) and fourth quarters (19.7 ± 9.0%).

Fc-receptors and immunophagocytosis

The percentage of Fc-receptor positive cells (73.0%, range 24.5 to 95.5%) did not change during the first year of CAPD treatment. There was no significant difference with the control peritoneal cells but relatively more macrophages were present in the control peritoneal cells. This implies that almost all CAPD peritoneal macrophages showed Fc-receptor positivity (irrespective of PI) and on average only 85% of the control peritoneal macrophages (P = 0.04). In general the CAPD peritoneal cells of HPI patients comprised a higher percentage of phagocytosing macrophages compared to LPI patients. This difference, however, was only significant in the last quarter of

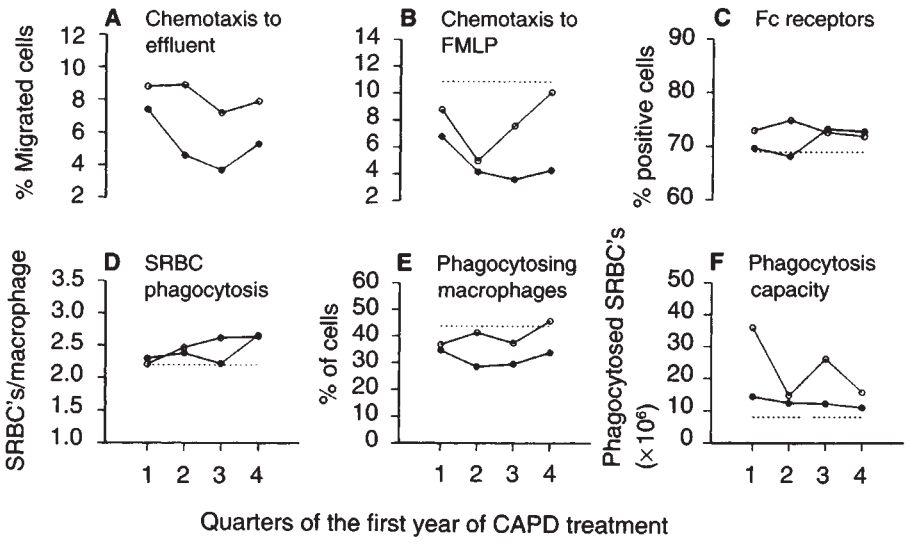

Fig. 5. *The immuno-effector characteristics of CAPD peritoneal cells.* The open symbols represent the high peritonitis incidence group (N = 19) and the closed symbols the low peritonitis incidence group (N = 37). The dashed line represents the laparoscopy cells.

the year (45.8 vs. 33.9%, P = 0.04). The number of [IgG]SRBC per phagocytosing CAPD peritoneal macrophage increased over the year (from 2.2 to 2.6, P = 0.04) in a similar way for both PI groups. The number of [IgG]SRBC per phagocytosing macrophages was closely related to the percentage of phagocytosing macrophages (r = 0.55, P < 0.001), but not to the percentage of Fc-receptor positive cells. The phagocytosis capacity decreased during the year (from 10.1 to 8.2, P = 0.01) but was similar to the phagocytosis capacity of the control cells (8.1 [IgG]SRBC's × 10^6) in every quarter of the year. The phagocytosis capacity was not related to the PI.

Discussion

In this study the peritoneal cellular immune system during the first year of CAPD treatment was investigated and compared to control peritoneal cells obtained by laparoscopy. Differences between the CAPD peritoneal cells and control peritoneal cells were found and marked changes related to duration of CAPD and/or PI were observed within this period. It should be realized that two basic assumptions underly the interpretations of the data from this study. First, it is assumed that the CAPD peritoneal cells from the overnight dialysis period reflect the actual immunological status of the peritoneal cavity during the whole day. Second, it is assumed that the CAPD peritoneal cells obtained from PDE reflect the total peritoneal cell population. This latter assumption is supported by a limited number of studies [25, 26] which suggest that a substantial population of peritoneal cells adhered to the peritoneum does not exist, as discussed by Holmes et al [27].

During the first year of CAPD treatment there was a gradual decrease in the yield of peritoneal cells. This may go on beyond the first year and stabilize at an average of 5 to 8 × 10^6 cells per overnight effluent after two to three years [12, 14, 27]. The decrease in CAPD peritoneal cells yield does not seem to be caused by a decrease in the chemo-attractivity of the PDE, since there was no decrease in the chemotaxis of healthy donor monocytes to PDE from the first and the fourth quarter. However, as dialysis fluid has been shown to cause an influx and activation of macrophages in the rat peritoneal cavity [28], it is proposed that the decrease in yield of CAPD peritoneal cells over time probably reflects the adaptation of the peritoneal cavity to the dialysis fluid by a mechanism still unknown.

In accordance with most studies [2, 3, 12, 14, 27, 29] the peritoneal macrophage was the predominant cell in the CAPD peritoneal cells and control peritoneal cells, although in some patients repeatedly more than 50% lymphocytes could be found in the CAPD peritoneal cells. The HPI patients had a significant higher percentage of macrophages (judged on morphological criteria and EBM11 positivity), especially during the first half year of treatment. Taken together with the total yield of peritoneal cells, there was also an absolute increase in peritoneal macrophages in HPI patients compared to LPI patients. This probably indicates a more stimulated peritoneal immune system in the HPI patient group. Most of the HPI patients (14 from the 19) had a peritonitis episode in the first month after starting CAPD, before their CAPD peritoneal cells had been analyzed. Therefore it was not possible to distinguish between cause and effect.

Mesothelial cells were only found in the CAPD peritoneal cells. The fresh CAPD fluid is cytotoxic for MC *in vitro* [30] and causes mesothelial hyperplasia after twice daily injection in the rat peritoneal cavity [31]. This implies that also in humans the mesothelium will be damaged during CAPD. Therefore the MC in the PDE reflect the active regeneration of the mesothelium, as has been discussed in a previous study [12]. This concept is supported by the finding that no MC were present in the control peritoneal cells. The importance of an intact mesothelium could be the production of prostaglandins [32, 33] or the prevention of bacterial adherence to submesothelial structures [34]. The percentage and yield of MC in HPI patients tended to be lower then in LPI patients but this difference did not reach statistical significance. Therefore these observations partly support the previous reports that showed a relation between a low yield or percentage of MC and a high PI [5, 12].

A high expression of HLA-DR/DQ was present on peritoneal macrophages of CAPD patients as well as of healthy controls. However, the CAPD peritoneal macrophages have a far better antigen presenting capacity when compared to monocytes and control peritoneal macrophages [24] indicating a functional

activation of the CAPD macrophages. Expression of HLA-DR/DQ was seen on all peritoneal macrophages irrespective of a HPI or a LPI. The percentage of macrophages is increased in HPI patients but this is not accompanied by a similar increase in the percentage of HLA-DR/DQ positive cells, as is seen for instance for the percentage of Fc-receptor positive cells. This indicates, although indirectly, that the percentage HLA-DR/DQ positive peritoneal lymphocytes in HPI patients is lowered when compared to LPI patients and therefore confirms the reported low percentage of HLA-DR positive peritoneal lymphocytes in HPI patients [1]. It has been suggested that this characteristic could be caused by a decreased secretion of IL-1 by peritoneal macrophages in such patients [11] and has been interpreted as evidence of a depressed peritoneal immune system. In this study it is shown that HPI patients have a general higher percentage of peritoneal macrophages, a higher chemotactic activity towards FMLP and a higher percentage of phagocytosing macrophages in the last quarter of the year (as will be discussed below), when compared to LPI patients. These observations indicate a more activated peritoneal immune system in HPI patients.

The CAPD peritoneal lymphocytes clearly showed features of an activated immune status by the presence of increased numbers of MHC class II positive lymphocytes when compared to control peritoneal cells. A decreased CD4/CD8 ratio (below 1.5) in comparison to the peritoneal blood is characteristic for the normal and CAPD peritoneal cavity [35, 36]. A relative increase of CD8 positive cells (T suppressor cells) over CD4 positive T cells (T helper cells) possibly plays an important role in parts of the body where activation of the local immune system is controlled [37]. The higher CD4/CD8 ratio in the CAPD peritoneal cavity when compared to the normal peritoneal cavity shows the relative increase of CD4 positive T cells in the CAPD peritoneal cavity. This shift in balance also indicates an activated CAPD peritoneal T cell system.

In the functional assays CAPD peritoneal cells only differed from control peritoneal cells in their increased percentage of Fc-receptor positive macrophages. In the rat peritoneal cavity dialysis fluid increases the percentage of Fc-receptor positive macrophages [28]. The finding of the relative higher percentage Fc-receptor-positive CAPD peritoneal macrophages therefore reflects a functional activation of the macrophages.

In contrast to others [2, 20], the results from the chemotaxis assay in general showed a lowered chemotactic activity toward FMLP for the CAPD peritoneal cells compared to the control peritoneal cells. However, it is important to distinguish between the chemotactic activity of the CAPD peritoneal cells of HPI and LPI patients, since the latter showed a lower response to FMLP and to the PDE. This is in accordance with Holmes et al [27] who reported a higher expression of C5a and FMLP receptors on the surface of peritoneal leukocytes of HPI patients, although this difference did not reach statistical significance. The chemotactic response to the PDE or FMLP remains at a high level for the CAPD peritoneal cells of HPI patients throughout the year whereas it decreases in LPI patients. This indicates that the peritoneal immune system stays at a higher level of activation in HPI patients.

An increase of phagocytosis activity but no increase in Fc-receptor positivity has been reported after an increase of dwell time [22, 38]. In this study and the study of Goldstein et al [2] the monocytes showed a higher chemotactic activity compared to the macrophages. Therefore the increase in the number of [IgG]SRBC phagocytosed per phagocytosing macrophages and the decrease of chemotactic activity in of CAPD peritoneal cells in LPI patients during the first year of CAPD treatment seem to be caused by an increased functional maturation stage of the CAPD peritoneal macrophages. However, these changes were not accompanied by an increase in the low percentage of RFD7 positive macrophages and the reasons for an accelerated maturation are not clear.

Due to the increase in [IgG]SRBC per phagocytosing macrophages the phagocytosis capacity of CAPD peritoneal cells changed little despite the sharp decrease in yield of CAPD peritoneal cells, and stayed at the level of control peritoneal cells. This is in accordance with our clinical experience with CAPD patients that the PI does not increase after prolonged CAPD treatment [39]. An increased percentage of phagocytosing macrophages in HPI patients was found in this study. Whereas the CAPD peritoneal cells of LPI patients tended to decrease the opposite was seen for HPI patients. This pattern is very similar to what is shown for the chemotactic activity and gives additional evidence to support the concept of a more activated peritoneal immune system in HPI patients. It is clear that this activation is not seen in every immunological parameter measured, and this could explain why others have not reported any relation between PI and immuno-effector functions of CAPD peritoneal cells [40, 41].

In conclusion, the CAPD peritoneal cells have features of both immaturity and activation when compared to control peritoneal cells. Changes in the yield and functional characteristics of CAPD peritoneal cells during the first year of CAPD treatment indicate an adaptation of the peritoneal immune system to an activating stimulus, perhaps caused by the dialysis fluid. No defects in immuno-effector functions could be detected in the CAPD peritoneal cells when compared to control peritoneal cells. A high number of peritonitis episodes caused a more activated peritoneal cellular immune system throughout the first year of CAPD and no significant risk factors that may contribute to a HPI could be identified.

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